

University of Groningen

Pre-zygotic isolation in the macroalgal genus *Fucus* from four contact zones spanning 100-10 000 years

Hoarau, Galice; Coyer, James; Giesbers, M.C.W.G.; Jüterbock, Alexander; Olsen, J.L.

Published in:
Royal Society Open Science

DOI:
[10.1098/rsos.140538](https://doi.org/10.1098/rsos.140538)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Hoarau, G., Coyer, J., Giesbers, M. C. W. G., Jüterbock, A., & Olsen, J. L. (2015). Pre-zygotic isolation in the macroalgal genus *Fucus* from four contact zones spanning 100-10 000 years: A tale of reinforcement? *Royal Society Open Science*, 2, [140538]. <https://doi.org/10.1098/rsos.140538>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Cite this article: Hoarau G, Coyer JA, Giesbers

MCWG, Jueterbock A, Olsen JL. 2015

Pre-zygotic isolation in the macroalgal genus

Fucus from four contact zones spanning

100–10 000 years: a tale of reinforcement?

R. Soc. open sci. **2**: 140538.

<http://dx.doi.org/10.1098/rsos.140538>

Received: 22 December 2014

Accepted: 13 January 2015

Subject Category:

Biology (whole organism)

Subject Areas:

evolution/ecology

Keywords:

introgression, hybridization, hybrid zones,
macroalgae, speciation

Author for correspondence:

G. Hoarau

e-mail: gah@uin.no

Electronic supplementary material is available

at <http://dx.doi.org/10.1098/rsos.140538> or via

<http://rsos.royalsocietypublishing.org>.

Pre-zygotic isolation in the macroalgal genus *Fucus* from four contact zones spanning 100–10 000 years: a tale of reinforcement?

G. Hoarau¹, J. A. Coyer², M. C. W. G. Giesbers³,
A. Jueterbock¹ and J. L. Olsen³

¹Faculty of Aquaculture and Biosciences, University of Nordland, Bodø 8049, Norway

²Shoals Marine Laboratory, Cornell University, 400 Little Harbor Road, Portsmouth, NH, USA

³Marine Benthic Ecology and Evolution Group, Centre for Ecological and Evolutionary Studies, University of Groningen, Nijenborgh 7, Groningen 9747 AG, The Netherlands

1. Summary

Hybrid zones provide an ideal natural experiment to study the selective forces driving evolution of reproductive barriers and speciation. If hybrid offspring are less fit than the parental species, pre-zygotic isolating barriers can evolve and strengthen in response to selection against the hybrids (reinforcement). Four contact zones between the intertidal macroalgae *Fucus serratus* (*Fs*) and *Fucus distichus* (*Fd*), characterized by varying times of sympatry and order of species introduction provide an opportunity to investigate reinforcement. We examined patterns of hybridization and reproductive isolation between *Fs* and *Fd* in: (i) northern Norway (consisting of two natural sites, 10 000 years old), (ii) the Kattegat near Denmark (*Fd* introduced, nineteenth century) and (iii) Iceland (*Fs* introduced, nineteenth century). Using 10 microsatellites and chloroplast DNA, we showed that hybridization and introgression decreased with increasing duration of sympatry. The two younger contact zones revealed 13 and 24% hybrids and several F_1 individuals, in contrast to the older contact zone with 2–3% hybrids and an absence of F_1 s. Cross-fertilization experiments revealed that the reduction in hybridization in the oldest zone is consistent with increased gametic incompatibility.

2. Introduction

The mechanisms of reproductive isolation and speciation are of central interest in evolutionary biology because of their role in determining gene flow between formerly interbreeding

populations [1,2]. The last decade has seen great progress in the study of individual components of reproductive isolation and provided insights into the genetic basis of particular isolating barriers [3,4]. Yet, reproductive isolation among most species pairs is not due to a single isolating factor, but is a consequence of a large number of different pre- and post-zygotic barriers [5,6] and their potentially complex interactions. In the case of hybridizing species, reproductive barriers are more or less permeable, allowing the sexual transfer of genetic material between species (i.e. introgression). Hybrid zones, therefore, provide a natural experiment to study the selective forces causing the evolution of reproductive barriers and ultimately, the mechanisms of speciation.

Hybrid zones exist in many forms: narrow or wide, ephemeral or long lasting, and linear or mosaic [7–10]. The structure of a hybrid zone and its evolutionary fate largely depend on the fitness of the hybrid individuals relative to the parental species and three general scenarios are possible [11]. First, if there is no selection against hybridization and introgression is extensive, all individuals become hybrids, creating a hybrid swarm or ‘extinction through hybridization’ [12,13]. Second, if introgressed individuals are genetically stabilized and/or colonize new habitats, novel evolutionary lineages can arise [14–17]. Finally, if hybrid offspring are less fit than the parental species, pre-zygotic isolating barriers can evolve and strengthen in response to selection against the hybrids (i.e. increase pre-zygotic reproductive isolation) in a process called reinforcement [18].

Despite a strong theoretical foundation, the importance of reinforcement in nature remains controversial [2,19]. Reinforcement may play a larger role in speciation than previously thought [20] as it can lead to speciation of sympatric populations that have been formerly allopatric [18] or it can lead to rapid allopatric speciation [21]. Documenting reinforcement in natural populations, however, remains challenging and must address several criteria [18], including occurrence of hybridization, selection against hybrids and displacement of a heritable trait perceived by the other sex. Nevertheless, reinforcement has been documented in an increasing number of vertebrates, insects and plants [21–39]. To our knowledge, no study has examined reinforcement in other eukaryote supergroups.

The intertidal seaweed genus *Fucus* (supergroup Heterokonta; Phaeophyta) is an ideal group in which to study speciation. The genus originated in the North Pacific and after the opening of the Bering Strait (5.5–5.4 Myr BP), colonized the North Atlantic where it radiated into two distinct lineages that diverged 0.9–2.25 Myr BP: lineage 1 including *Fucus distichus*, *Fucus serratus*; and lineage 2 including *Fucus spiralis*, *Fucus vesiculosus* and others [40–45]. Each lineage is characterized by sister taxa that are dioecious with unisexual (male or female) conceptacles (e.g. *F. serratus* and *F. vesiculosus*) and hermaphrodites with co-sexual conceptacles (e.g. *F. distichus* and *F. spiralis*). The dioecy/hermaphroditism character has undergone multiple-state changes during the evolution of the genus *Fucus* [40,42,44]. Hybridization is common within the genus and molecular studies have shown that hybridization is prevalent among sister taxa (i.e. within one lineage) and mostly involves one dioecious and one hermaphrodite parental species (e.g. *F. serratus* × *F. distichus* [46,47] and *F. vesiculosus* × *F. spiralis* [48–52]). A distinguishing feature of *Fucus* hybrid zones is the limited gamete dispersal, because egg-produced pheromones effectively attract sperm only within distances of micrometres to millimetres [53] and settlement of fertilized eggs most often occurs within 1–2 m of the parent [54,55].

Lineage 1 consists of the dioecious *F. serratus* (hereafter *Fs*) with a temperate east Atlantic distribution. The sister species *F. distichus* (hereafter *Fd*) is a hermaphrodite and characterized by an Arctic distribution (figure 1). The two species diverged 2.25 to 0.9 Myr ago [40,41]. Although several species have been described within the *F. distichus* lineage on the basis of morphological characteristics (e.g. [56,57]), current molecular phylogenetic evidence indicates that none form monophyletic groups and, therefore, from a phylogenetic standpoint, all are presently considered part of a single species [40,58].

Four contact zones involving *Fs* and *Fd* were identified (figure 1): (i) two in Northern Norway where the two species have been in sympatry for *ca* 10 000 years after the ice receded from the area since the Last Glacial Maximum (LGM) and *Fs* recolonized the Norwegian coast from a refugium in southwest Ireland [41]; *Fd*, on the other hand, appears to have survived the LGM in northern Norway based on mtDNA and microsatellite data [59]; (ii) Iceland (Heimaey), where *Fs* was introduced from Oslofjord (Norway), probably via imported raw wood logs, to an endemic population of *Fd* [60], leading to *ca* 100 years of sympatry; and (iii) the Kattegat Sea, where *Fd* (formerly *Fucus evanescens*) was introduced in the mid-1890s to an endemic *Fs* population [61,62], also leading to *ca* 100 years of sympatry; however, the source has not been identified owing to the lack of variability at nuclear markers (G. Hoarau and J. A. Coyer 2010, unpublished data). Crucially, the reproductive seasons of each species overlap in all zones of sympatry.

The identified contact zones between *Fs* and *Fd*, with varying times of sympatry and directionality of introduction (i.e. *Fs* to *Fd* or *Fd* to *Fs*), provide a unique opportunity to investigate the presence of

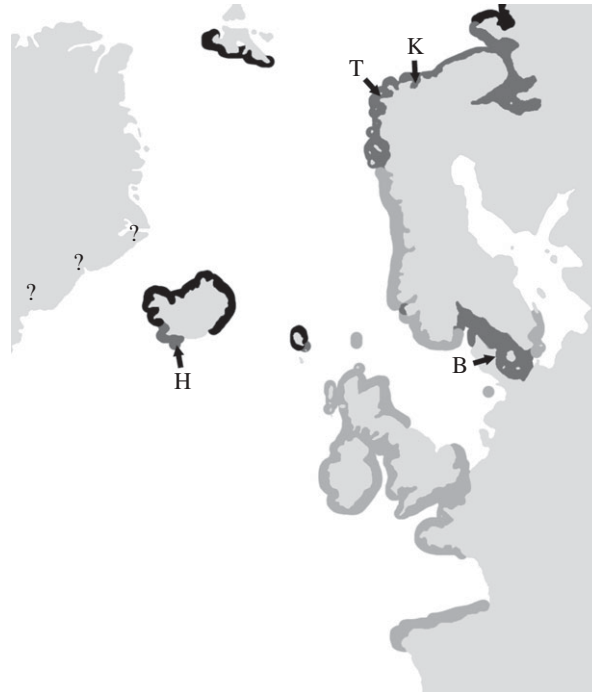


Figure 1. Sampling locations for *Fucus serratus* and *Fucus distichus*. Light grey depicts *F. serratus* distribution, black *F. distichus* distribution in the northeast Atlantic and dark grey sympatry. Distribution along the Greenland coast is uncertain and is designated with '?'.

reinforcement. Reinforcement is most likely to occur in areas where there is strong selection against hybrids and restricted gene flow [19], both of which are characteristic of the *Fs/Fd* hybrids zones [46,47]. Furthermore, as the evolution of pre-zygotic isolating barriers is expected to result in a reduction of interspecific mating through time [35], reinforcement will be stronger in older zones of sympatry. Specifically, populations in northern Norway (10 000 years of contact) should show a lower degree of hybridization and interspecific fertilization success relative to the younger hybrid zones (100 years) in the Kattegat and in Iceland.

In this study, we examined patterns of hybridization and reproductive isolation between *Fs* and *Fd* in two young and two old contact zones. Specifically, we: (i) determined the degree of hybridization and introgression using blind sampling with subsequent identification of parents and hybrids using both nuclear and chloroplast markers, and (ii) compared the degree of interspecific fertilization success within and across contact zones.

3. Material and methods

3.1. Sampling

Hybridization among *Fs* and *Fd* was examined at all contact zones: (i) Kirkenes ($n = 47$) and Tromsø (northern Norway) ($n = 192$), where both species are native and have been in sympatry for *ca* 10 000 years; (ii) Heimaey (Iceland) ($n = 96$), where *F. serratus* was introduced *ca* 100 years ago; and (iii) Blushøj (Kattegat, Denmark) ($n = 286$), where *Fd* was introduced *ca* 120 years ago. At both Tromsø and Blushøj, two 2×12 m plots were established to investigate micro-scale differences in hybridization, with the long axis perpendicular to the shoreline (beginning at the shallowest appearance of *Fd* and finishing at the deepest occurrence of *F. serratus*). Owing to physical constraints at Kirkenes and Heimaey, only one plot was established. Within each plot, a small tissue sample was excised *in situ* from all post-recruit (more than 10 cm) individuals without *a priori* species identification. Tissues were stored in silica gel crystals prior to genetic analysis. All data from Blushøj are from Coyer *et al.* [46].

3.2. DNA extraction and microsatellite analysis

DNA was extracted from *ca* 5 mg of silica-dried tissue as described in Hoarau *et al.* [41]. We used 10 microsatellite loci: FsA198, FsB113, FsB128, FsD39, L20, L38, L58, L94, FeF172 and FsF4 from Coyer

et al. [46]. PCR reaction mixtures and conditions are described in Coyer *et al.* [46]. All genotypes were visualized on an ABI 377 or on a 3500XL automatic sequencer (Life technologies) and analysed with GENESCAN software (Life technologies).

3.3. Chloroplast marker

We used a portion of the ribulose-1,5-bisphosphate carboxylase operon (Rubisco; chloroplast) to identify the egg-producing parent for hybrids, as the parental species differed by a 33 bp indel [63]. PCR reactions and conditions are described in Coyer *et al.* [63] and parental identity was detected by sizing of PCR products on an ABI 377 or on a 3500XL automatic sequencer (Life technologies).

3.4. Molecular data analysis

Genetic diversity using Nei's [64] non-biased H_{exp} , estimators of F_{IS} and F_{ST} (Wright [65]) as f and θ , [66], and allele frequencies were estimated using the software GENETIX 4.02 [67]. Multilocus heterozygosity (MLH) was defined as the number of heterozygous loci per individual and ranged from 0 to 10.

Admixture of microsatellite genotypes was analysed with STRUCTURE [68], which uses a Bayesian approach to identify K user-defined clusters of individuals that are genetically homogeneous. Sampled individuals were assigned either to clusters or jointly to two or more clusters if their genotypes indicated admixture. All analyses were replicated 10 times for each location independently (table 1) to ensure proper convergence of the Markov chain Monte Carlo (MCMC) with the parameters: ancestry model = admixture (to account for recent divergence and shared ancestral polymorphisms); frequency model = independent; burn-in = 1 000 000; MCMC length = 2 000 000 post-burn-in and $K = 2$ (to account for the parental species). The analysis with STRUCTURE produced for each individual, an admixture coefficient (I_{FS}) defined as the proportion of membership to the *F. serratus* cluster.

The power of admixture analyses to detect hybridization in *Fucus* was evaluated by simulation using HYBRIDLAB (v. 1.0) [69] as described previously [46]. Simulations were repeated 10 times for each location independently and these results were used to define the boundaries of the I_{FS} admixture coefficient (95% confidence intervals) for: (i) pure *F. serratus*, (ii) pure *F. distichus*, and (iii) F_1 hybrids.

3.5. Fertilization success (pre-zygotic isolation)

Reproductive receptacles (consisting of 100s of conceptacles) from both species were collected within each of the contact zones (except Kirkenes). Receptacles were packed individually in aluminium foil and transported to the laboratory in a cooler box within 36–48 h. A reciprocal crossing design was used for Blushøj, Tromsø and Heimaey, using five *Fd* females, five *Fs* males, five *Fs* females and five *Fd* males, resulting in 25 *Fd* conspecific crosses, 25 *Fs* conspecific crosses and 2×25 interspecific crosses. Additionally, negative controls consisting of eggs only (no sperm added) were used for all females. All specimens were genotyped with 10 microsatellite loci and analysed as described above (e.g. STRUCTURE) to determine species identity.

Crosses were performed in sterile plastic culture plates containing 3 ml sterile seawater at 5°C and each cross and control was replicated four times using 10 oogonia (80 eggs) in each replicate. *Fs* is a dioecious species, with receptacles from a mature individual possessing conceptacles containing either antheridia or oogonia. The sex of *Fs* individuals was determined microscopically. *Fs* receptacles were stored overnight at 4°C. The addition of ice-cold sterilized seawater induced conceptacles to release eggs and sperm, which were collected and washed once in sterile seawater before further use.

As *Fd* is a hermaphroditic species with individual conceptacles within the receptacle containing both antheridia and oogonia, collecting eggs after release from conceptacles was inappropriate, because eggs from such preparations were potentially already fertilized by conspecific sperm. To prevent self-fertilization during gamete extraction from *Fd*, eggs were collected with a pipette immediately after sectioning receptacles with a razor blade in order to disrupt the conceptacles and then sequentially washed ($\times 3$) in 10 ml sterile seawater to dilute any accompanying sperm and minimize/eliminate self-fertilization. Sperm from *Fd* was also obtained by sectioning as previously described, but sections were immediately placed in a small mesh bag (50 μm mesh size) that allowed free passage of the small sperm (5–6 μm dia.), but retained the larger eggs (70–100 μm dia.). The bags were then placed in a culture dish previously inoculated with eggs for 2 h. Fertilization success was evaluated after 48 h and confirmed after one week with the development to embryos. Successes of the various combinations were compared using ANOVA and Tukey's post hoc test.

Table 1. Summary of conditions and results for old and young $F_5 \times F_d$ hybrid zones.

location	contact zone (time, years)	total no. samples	total % of hybrids (figure 2)	% of F_1 hybrids	F_{ST} pure F_d versus pure F_5 (all $p < 0.001$)	genetic diversity (H_{exp})	$F_d F_{IS}$ (95% CI)	interspecific fertilization success (pre-zygotic)	F_1 fitness (post-zygotic)
Kirkenes NO natural	$\approx 10\,000$	47	2.1	0	0.60	F_d 0.25 F_5 0.33	0.78 (0.69–0.86)	not tested	no F_1 present
Tromsø NO natural	$\approx 10\,000$	192	3.1	0	0.47	F_d 0.17 F_5 0.52	0.68 (0.58–0.76)	0–1.1%	no F_1 present
Heimaey IC F_5 introduced	≈ 100	96	23.9	8.3	0.69	F_d 0.10 F_5 0.28	0.74 (0.56–0.88)	23.7–38.3%	lower fertility survivorship not tested
Blushøj DK ^a F_d introduced	≈ 100	286	12.9	3.7	0.63	F_d 0.12 F_5 0.50	0.77 (0.69–0.84)	9.6–43.1%	lower fertility lower survivorship

^aData from [46].

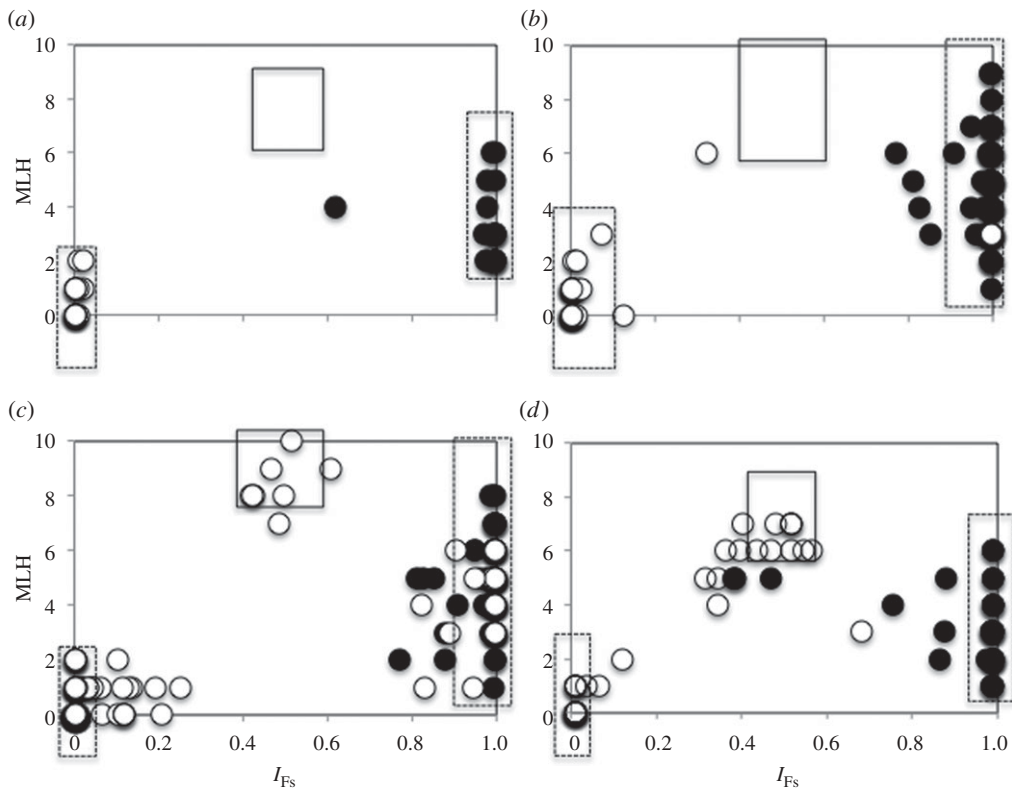


Figure 2. Relationship between individual MLH and introgression (I_{FS}) for the ‘old’ ((a,b) Kirkenes and Tromsø) and the ‘young’ contact zones ((c,d) Blushøj and Heimaey). Boxes depict results from the simulation analysis: stippled = pure species; dark grey = F_1 hybrids. Individuals were further classified by the origin of their chloroplasts: open circles = *F. distichus*, filled circles = *F. serratus*.

4. Results

4.1. Molecular characterization of the hybrid zones

The number of hybrids did not differ between plots within Tromsø and within Blushøj (data not shown); hence, data from both plots were subsequently pooled. Differentiation between parental species was strong with the highest F_{ST} estimates in the two younger contact zones (Heimaey 0.69, Blushøj 0.63) compared to the two locations in the older contact zones (Kirkenes 0.60; Tromsø 0.47) (all $p < 0.001$). In all four locations, the parental species showed different levels of genetic diversity with the highest level of heterozygosity in the dioecious *Fs* and the lowest in hermaphroditic *Fd* (table 1). Heterozygote deficiencies (F_{IS}) were equally high for *Fd* at all four locations (table 1).

In the oldest contact zones (Kirkenes and Tromsø locations), no F_1 hybrids were found and introgressed individuals accounted for only 2.1% of the 47 individuals and 3.1% of the 192 individuals, respectively (table 1 and figure 2). By contrast, 8.3% and 3.7% F_1 hybrids, and 23.9% and 12.9% introgressed individuals were found in the youngest contact zones (Heimaey and Blushøj, respectively). All F_1 and almost all hybrids (53 out of 68) were characterized by an *Fd* chloroplast (figure 2). In both Tromsø and Blushøj, several individuals with an *Fs* microsatellite genotype possessed an *Fd* chloroplast, but the reverse was never found.

4.2. Fertilization success

Conspecific fertilization successes (both *Fd* × *Fd* and *Fs* × *Fs*) did not differ significantly among the three contact zones examined (Kirkenes not tested) (67–49%, $p = 0.1199$) (figure 3). In the young contact zones at Heimaey and Blushøj, interspecific fertilization successes involving *Fd* egg and *Fs* sperm were comparable to the conspecific success (38% and 43%, respectively), but the interspecific fertilization successes involving *Fs* egg and *Fd* sperm were significantly lower than the conspecific crosses (24% and

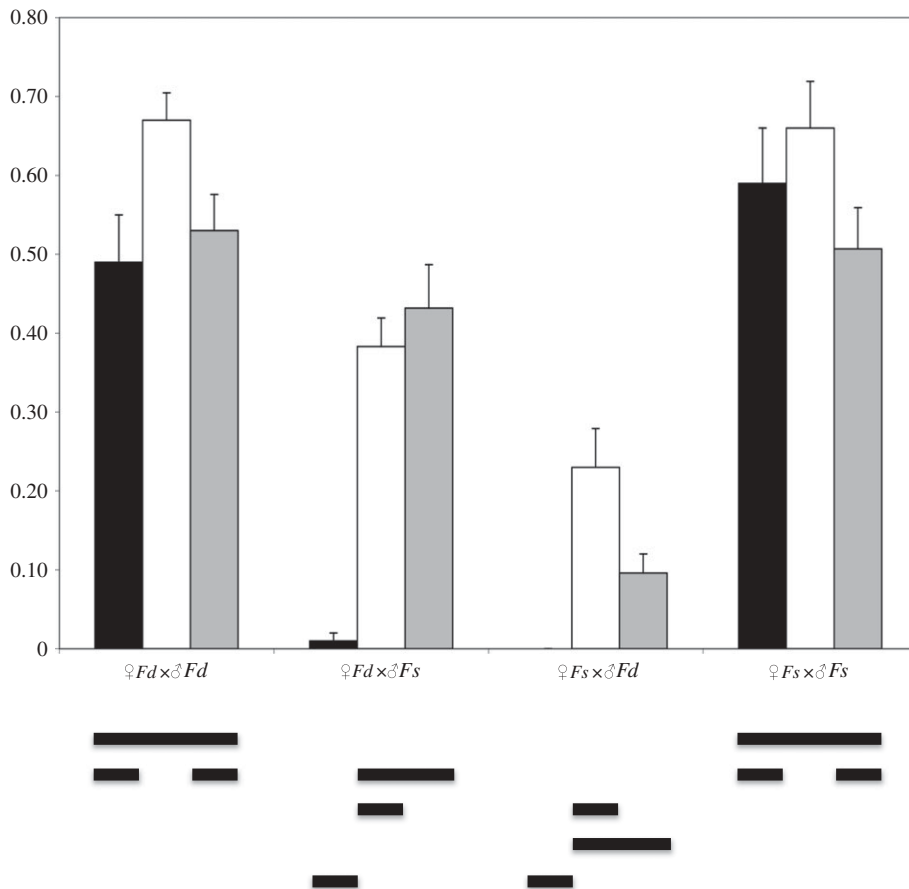


Figure 3. Fertilization success (the presence of one-week-old embryos) for all combinations of laboratory crosses (sample size = 100). Black = Tromsø, white = Heimaey, grey = Blushøj, Kirkenes not tested. Means grouped by a horizontal line are not significantly different from each other (Tukey post hoc, $p > 0.05$).

10%, respectively, $p < 0.001$) (figure 3). In sharp contrast, both interspecific crosses showed significantly ($p < 0.001$) lower success (less than 1.1%) in the oldest contact zone at Tromsø (Kirkenes not tested).

5. Discussion

Natural selection can drive speciation by ‘reinforcing’ those mechanisms preventing hybridization when hybrids are maladaptive. As maladaptive hybrids can be viewed as energetically expensive ‘mistakes’, pre-zygotic mechanisms may evolve to prevent hybridization. These mechanisms range from preventing mating behaviour (through enhancement of species recognition systems) to preventing gamete recognition (fertilization). In particular, pheromones and gamete surface receptor polysaccharides have been reported from a wide variety of eukaryotic species (see [33,35] and references therein) including the brown algae (fucoserrate in *F. vesiculosus* and *F. serratus*) [70]. However, these recognition molecules have primarily been studied in the context of gamete attraction rather than as possible pre-zygotic isolation mechanisms.

The pattern of greater pre-zygotic isolation in sympatry relative to allopatry is one of the main signatures of reinforcement and has been termed reproductive character displacement’ [19,36]. However, establishing an empirical case for reinforcement remains difficult because isolation is not necessarily due to a single isolating factor, but a consequence of several pre- and (possibly) post-zygotic barriers and their interactions. Nevertheless, a number of criteria have been proposed to demonstrate reinforcement [18,33] including heterospecific mating (gene flow between parental species), presence of maladaptive hybrids, frequency of hybridization between populations across time, reproductive character displacement, and the absence of alternative explanations (e.g. ecological divergence via local selection or temporal isolation).

The $Fd \times Fs$ hybrid zones we examined meet some of the reinforcement criteria. First, hybridization is occurring or has occurred in all contact zones. Both younger contact zones show a high percentage of hybrids (13–24%) and several F_1 individuals. Despite low contemporary hybridization in the older contact zone, there is evidence that hybridization has occurred in the past, as ca 3% of the individuals genotyped in the ‘old’ contact zones carry alleles from the other species (table 1 and figure 2). Furthermore, one individual with a pure Fs nuclear genotype and a Fd chloroplast DNA was found in Tromsø, resulting from past introgression.

Second, hybridization between Fs and Fd appears to be maladaptive. the Blushøj F_1 hybrids showed a lower fitness compared with the parental species, with selection coefficients against hybrids ca 80% [46] and reproductive F_1 hybrids showed reduced fertility, mostly owing to lower egg quality (e.g. fewer and more variably sized eggs per receptacle). Additionally, laboratory crosses with eggs from F_1 hybrids were significantly less successful both in Blushøj [47] and Heimaey where lower egg quality was also observed (J. A. Coyer and G. Hoarau 2002, unpublished data). Survivorship and fertility of F_1 hybrids were not assessed at Tromsø and Kirkenes as no F_1 hybrids were found in the field. In *Fucus*, as in several other species, reduction of fertility and potential sterility appear before hybrid inviability [12,71,72].

Third, patterns observed in the $Fd \times Fs$ hybrid zones are consistent with the presence of reproductive character displacement. In contrast to virtually all previous studies of reinforcement, which have compared populations of plants and animals in regions of sympatry to those in allopatry, we examined sympatric populations differing in the time of sympatry (100 to 10 000 years), as reinforcement is expected to result in a reduction of interspecific mating through time ([35] and references therein). Our molecular data suggest that hybridization has decreased with the time of contact. Although based on only two locations per age, both of the younger contact zones revealed a high percentage of hybrids (13–24%) and the presence of F_1 individuals. By contrast, hybridization was very low (2–3%) and no F_1 individuals were found in either of the old contact zones in northern Norway (table 1 and figure 2). Our cross-fertilization experiments further suggested that the reduction in hybridization observed in Tromsø was the result of increased gametic incompatibilities. Whereas interspecific fertilization success (Fd egg and Fs sperm) was comparable to conspecific crosses in the younger contact zones, interspecific fertilization successes decreased to virtually zero in Tromsø (figure 3). While our results cannot provide conclusive evidence for reinforcement (i.e. increased isolation in sympatry compared with allopatry using the temporal comparison) because of low replicates, they are consistent with patterns that would be expected over such a period of time.

In this context, we reviewed a new contact zone in Bergen Harbour, where Fd was introduced to Fs within the past 10 years, where we expected to see many hybrids. Unexpectedly, a genetic survey conducted in 2008 found no hybrids (G. Hoarau and J. A. Coyer 2008, unpublished data). At least three aspects of the Bergen population, however, can temper the contradiction. First, the source of Fd to Bergen Harbour is unknown and to date, impossible to trace (G. Hoarau and J. A. Coyer 2008, unpublished data). The source of Fd in Bergen could be northern Norway and subsequently, a population already displaying strong reinforcement. Second, the phenological overlap in Bergen Harbour is reduced (K. Sjøtun 2008, personal communication) compared with all other zones of contact, thus limiting the temporal window for hybridization. This difference in observed phenology at Bergen, in fact, suggests a northern origin of Fd [73]. Finally, *F. distichus* is an order of magnitude more abundant than *F. serratus* in Bergen Harbour (J. A. Coyer, K. Sjøtun and G. Hoarau 2008, unpublished data), thus further limiting the likelihood of hybridization (which requires threshold densities of gametes). These results further highlight the difficulties in definitively proving reinforcement. Nevertheless, we consider the temporal approach involving contact zones compelling (especially, the older ones).

Ecological divergence caused by strong local selection is also a potentially contributing mechanism, especially when gamete recognition systems are weak (reviewed in [74], see also [75,76]). Although Fd and Fs can differ in position on the intertidal shore, with Fd occurring slightly higher on the shore than Fs , wide areas of intermixing were the rule at all four contact zones and our sampling occurred within the areas of intermixing (table 1). Furthermore, no pattern of microhabitat utilization was detected in a detailed spatial analysis of the parental species and F_1 hybrids at Blushøj: mature plants were intermingled and F_1 hybrids occurred throughout the intermingled area rather than clustered around either parental species or themselves [46]. Similar observations were made at Tromsø (J. A. Coyer and G. Hoarau 2010, unpublished data). Nevertheless, microhabitat differentiation cannot be entirely ruled out. In addition, no phenological differences were apparent as reproductive seasons of both parental species overlapped in all zones of sympatry and sexually mature individuals of both species were

abundant when we sampled (G. Hoarau and J. A. Coyer 2010, personal observation); contrary to the situation in Bergen.

Changes in the mating system, such as increased/decreased self-fertilization in plants, can be another mechanism for reproductive character displacement (reviewed in [36]). Selfing may thus promote pre-zygotic isolation. In the hermaphroditic *Fd*, high inbreeding coefficients (up to $10\times$ higher than *Fs*) have been routinely found in broad surveys (Coyer *et al.* [59]), whereas in the dioecious *Fs*, this is seldom the case (Coyer *et al.* [47]). However, no evidence for mating system change was observed, as inbreeding coefficients (F_{IS}) for *Fd* were not significantly different among the contact zones (table 1) and increased levels of self-fertilization did not occur. As shown in this study, as well as Coyer *et al.* [46], hybridization is asymmetrical with *Fd* providing the egg most of the time (figure 3). To our knowledge, no model of hybrid zones has considered a mixed mating system and little more can be said at the present time. What we do know, however, is that hybrids are almost never formed between *Fucus* species with the same mating systems.

We hypothesized that sexual trait(s) accounting for reinforcement in *Fucus* would most likely occur at the level of gamete attraction and/or recognition. Gamete recognition proteins/genes in marine organisms have been investigated mainly in marine invertebrates (mussels, snails and sea urchins) [76,77]. In these systems, positive selection (as assessed by high sequence divergence and more non-synonymous than synonymous substitutions) for sperm-egg interaction proteins has been found in closely related and sympatric species of sea urchins [78,79], but not for species in allopatry [80]. Sympatry among closely related species also seems to be associated with increased sperm specificity. In sea urchins, for example, crosses between allopatric species pairs require substantially less sperm for fertilization than crosses involving sympatric pairs [81]. The sperm binding protein *fs27* in *Fucus* has long been known and shares a number of chemical properties with sea urchin bindin gamete recognition protein [82–84]. Recognition between eggs and sperm has been hypothesized to be based upon a receptor mechanism involving ligands (oligosaccharide side-chains of egg surface glycoproteins) and complementary binding proteins [84]. However, the protein and its ligand remain to be sequenced.

Another important evolutionary mechanism potentially occurring at the level of gamete attraction/recognition is sexual conflict [85,86]. For example, an overabundance of sperm increases the risk of polyspermy (multiple sperm entry), which will prevent subsequent development of embryos. Under these conditions, male and female gametes have different priorities: males for fast entry into eggs, females for prevention of polyspermy and slow sperm entry. Any mutation that makes it more difficult to fertilize eggs can concomitantly lower the effective concentration of sperm, thereby allowing eggs to block polyspermy. Thus, a conflict over fertilization rates arises in which females are selected for lower egg–sperm affinity, whereas males are selected for higher affinity. This affinity ‘arms race’ could lead to divergences in the gamete recognition system and thus to reproductive character displacement, independent from reinforcement [76,87].

Fucus species display a fast and sodium-dependent block to polyspermy [88,89]. Consequently, polyspermy rates in natural populations are higher in brackish (*F. vesiculosus* [90]) relative to fully marine habitats (*F. ceranoides* [91], *F. distichus* [92]). Three of the contact zones examined in this study (Tromsø, Kirkenes and Heimaey) were fully marine and although the Blushøj site could vary between near fully marine (30 psu) to semi-brackish (20 psu) over a time scale of hours to days, polyspermy in *F. vesiculosus* most commonly occurs at less than 6.5 psu [90]. Consequently, polyspermy is unlikely to drive sexual conflict among *Fd* and *Fs* gametes in the contact zones we examined. An additional outcome of divergence from sexual conflict is lower conspecific fertilization success (reviewed in [36]), which was not observed in our study. Therefore, the most parsimonious explanation for the pattern we observed in *Fd* \times *Fs* hybrid zones is probable reinforcement of pre-zygotic isolation mechanisms.

6. Conclusion

The genus *Fucus*, with the combination of contemporary radiation (e.g. *F. radicans* within the past 400–2000 years; [93], secondary contact zones (e.g. Coyer [46]), extensive hybridization (e.g. [46,50]), and a well-supported phylogenetic [40,42,44] and phylogeographic framework [18,19], e.g. *F. serratus* [41], e.g. *F. distichus* [58], e.g. *F. vesiculosus* and *F. spiralis* [43]) provides evolutionary biologists with a system phylogenetically distant from, but complementary to, plants and animals. While the results of our study of *Fd* \times *Fs* hybrid zones do not prove reinforcement, they are consistent with reinforcement of pre-zygotic isolation as shown by the decreasing rates of hybridization and interspecific fertilization success with increasing time of sympatry. Evidence for reinforcement will be most compelling when observed

in species pairs with a well-corroborated historical biogeographical framework, as it the case in *Fucus* (e.g. [21]).

Data accessibility. The supporting data can be found in the electronic supplementary material.

Acknowledgements. We gratefully acknowledge E. Boon, K. Gunnarsson, H. Halldórsson, G. V. Helgason, A. Peters, I. Smolina and R. Sveinsson for assistance in the field and B. D. H. K. Eriksson for assistance with statistical analyses.

Author contributions. G.H. designed the study, collected the samples, participated in the molecular work, analysed the data and drafted the manuscript, J.A.C. collected the samples, participated in the molecular work and the data analysis, and helped draft the manuscript, M.C.W.G.G. participated in the molecular work and the data analysis, A.J. collected samples, participated in the molecular work and the data analysis, J.L.O. coordinated the study and helped draft the manuscript.

Funding statement. The research was supported in part by the *Nederlands Wetenschappelijke Organisatie* (NWO) VENI 863.05.008 (G.H.), the IHP (Improving Human Potential) Programme of the European Commission (Iceland) (J.A.C.), the Norwegian Research Council HAVKYST 196505 & 203839 (G.H.) and KNAW Schure-Beijerinck-Popping Funds SBP/JK2006–46 (G.H.), SBP/JK2007–12 (J.A.C.) and SBP/JK2008–22 (G.H.).

Competing interests. We declare we have no competing interests.

References

- Butlin R *et al.* 2011 What do we need to know about speciation? *Trends Ecol. Evol.* **27**, 1–39.
- Abbott R *et al.* 2013 Hybridization and speciation. *J. Evol. Biol.* **26**, 229–246. (doi:10.1111/j.1420-9101.2012.02599.x)
- Hoballah ME, Gubitz T, Stuurman J, Broger L, Barone M, Mandel T, Dell'Olivo A, Arnold M, Kuhlmeier C. 2007 Single gene-mediated shift in pollinator attraction in *Petunia*. *Plant Cell* **19**, 779–790. (doi:10.1105/tpc.106.048694)
- Bomblies K, Weigel D. 2007 Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nat. Rev. Genet.* **8**, 382–393. (doi:10.1038/nrg2082)
- Coyne JA, Orr HA. 2004 *Speciation*. Sunderland, MA: Sinauer Associates.
- Rieseberg LH, Willis JH. 2007 Plant speciation. *Science* **317**, 910–914. (doi:10.1126/science.1137729)
- Barton NH, Hewitt GM. 1985 Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* **16**, 113–148. (doi:10.1146/annurev.es.16.110185.000553)
- Bull CM. 1991 Ecology of parapatric distributions. *Annu. Rev. Ecol. Syst.* **22**, 19–36. (doi:10.1146/annurev.es.22.110191.000315)
- Harrison RG, Rand DM. 1989 Mosaic hybrid zones and the nature of species boundaries. In *Speciation and its consequences* (eds D Otte, JA Endler), pp. 111–133. Sunderland, MA: Sinauer.
- Hewitt GM. 1989 The subdivision of species by hybrid zones. In *Speciation and its consequences* (eds D Otte, JA Endler), pp. 85–110. Sunderland, MA: Sinauer.
- Seehausen O. 2004 Hybridization and adaptive radiation. *Trends Ecol. Evol.* **19**, 198–207. (doi:10.1016/j.tree.2004.01.003)
- Coyne JA, Orr HA. 1998 The evolutionary genetics of speciation. *Phil. Trans. R. Soc. Lond. B* **353**, 287–305. (doi:10.1098/rstb.1998.0210)
- Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow JL. 2006 Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. *Mol. Ecol.* **15**, 343–355. (doi:10.1111/j.1365-294X.2005.02794.x)
- Arnold ML, Hodges SA. 1995 Are natural hybrids fit or unfit relative to their parents? *Trends Ecol. Evol.* **10**, 67–71. (doi:10.1016/S0169-5347(00)88979-X)
- Gardner JPA. 1997 Hybridization in the sea. *Adv. Mar. Biol.* **31**, 1–65. (doi:10.1016/S0065-2881(08)60221-7)
- Lowe A, Harris S, Ashton P. 2004 *Ecological genetics: design, analysis, and application*. Oxford, UK: Blackwell.
- Mallet J. 2005 Hybridization as an invasion of the genome. *Trends Ecol. Evol.* **20**, 229–237. (doi:10.1016/j.tree.2005.02.010)
- Howard DJ. 1993 Reinforcement: origin, dynamics and fate of an evolutionary hypothesis In *Hybrid zones and the evolutionary process* (ed. RG Harrison), pp. 46–69. Oxford, UK: Oxford University Press.
- Servedio MR, Noor MAF. 2003 The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Syst.* **34**, 339–364. (doi:10.1146/annurev.ecolsys.34.011802.132412)
- Smadja C, Butlin R. 2006 Speciation: a new role for reinforcement. *Heredity* **96**, 422–423. (doi:10.1038/sj.hdy.6800826)
- Hoskin CJ, Higgie M, McDonald KR, Moritz C. 2005 Reinforcement drives rapid allopatric speciation. *Nature* **437**, 1353–1356. (doi:10.1038/nature04004)
- Noor MA. 1995 Reinforcement in speciation. *Trends Ecol. Evol.* **10**, 492. (doi:10.1016/S0169-5347(00)89204-6)
- Saetre GP, Moum T, Bures S, Kral M, Adamjan M, Moreno J. 1997 A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* **387**, 589–592. (doi:10.1038/42451)
- Rundle HD, Schluter D. 1998 Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* **52**, 200–208. (doi:10.2307/2410935)
- Higgie M, Chenoweth S, Blows MW. 2000 Natural selection and the reinforcement of mate recognition. *Science* **290**, 519–521. (doi:10.1126/science.290.5491.519)
- Pfennig KS, Simovich MA. 2002 Differential selection to avoid hybridization in two toad species. *Evolution* **56**, 1840–1848. (doi:10.1111/j.0014-3820.2002.tb00198.x)
- Pfennig KS. 2003 A test of alternative hypotheses for the evolution of reproductive isolation between spadefoot toads: support for the reinforcement hypothesis. *Evolution* **57**, 2842–2851. (doi:10.1111/j.0014-3820.2003.tb01525.x)
- Nosil P, Crespi BJ, Sandoval CP. 2003 Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proc. R. Soc. Lond. B* **270**, 1911–1918. (doi:10.1098/rspb.2003.2457)
- Smadja C, Ganem G. 2005 Asymmetrical reproductive character displacement in the house mouse. *J. Evol. Biol.* **18**, 1485–1493. (doi:10.1111/j.1420-9101.2005.00944.x)
- Jaenike J, Dyer KA, Cornish C, Minhas MS. 2006 Asymmetrical reinforcement and *Wolbachia* infection in *Drosophila*. *PLoS Biol.* **4**, 1852–1862. (doi:10.1371/journal.pbio.0040325)
- Kronforst MR, Young LG, Gilbert LE. 2007 Reinforcement of mate preference among hybridizing *Heliconius* butterflies. *J. Evol. Biol.* **20**, 278–285. (doi:10.1111/j.1420-9101.2006.01198.x)
- Nosil P, Crespi BJ, Gries R, Gries G. 2007 Natural selection and divergence in mate preference during speciation. *Genetica* **129**, 309–327. (doi:10.1007/s10709-006-0013-6)
- Kay KM, Schemske DW. 2008 Natural selection reinforces speciation in a radiation of neotropical rainforest plants. *Evolution* **62**, 2628–2642. (doi:10.1111/j.1558-5646.2008.00463.x)
- Urbanelli S, Porretta D. 2008 Evidence of reinforcement of premating isolation between two species of the genus *Ochthebius* (Coleoptera: Hydraenidae). *Evolution* **62**, 1520–1527. (doi:10.1111/j.1558-5646.2008.00381.x)
- Lemmon EM, Lemmon AR. 2010 Reinforcement in chorus frogs: lifetime fitness estimates including intrinsic natural selection and sexual selection against hybrids. *Evolution* **64**, 1748–1761. (doi:10.1111/j.1558-5646.2010.00955.x)
- Ortiz-Barrientos D, Grealis A, Nosil P. 2009 The genetics and ecology of reinforcement implications for the evolution of prezygotic isolation in sympatry and beyond: the year in evolutionary biology. *Ann. NY Acad. Sci.* **1168**, 156–182.
- Peterson MA, Honchak BM, Locke SE, Beaman TE, Mendoza J, Green J, Buckingham KJ, White MA, Monsen KJ. 2005 Relative abundance and the species-specific reinforcement of male mating preference in the *Chrysomelidae* hybrid zone. *Evolution* **59**, 2639–2655. (doi:10.1111/j.0014-3820.2005.tb00976.x)
- Hopkins R. 2013 Reinforcement in plants. *New Phytol.* **197**, 1095–1103. (doi:10.1111/nph.12119)

39. Yukilevich R. 2012 Asymmetrical patterns of speciation uniquely support reinforcement in *Drosophila*. *Evolution* **66**, 1430–1446. (doi:10.1111/j.1558-5646.2011.01534.x)
40. Coyer JA, Hoarau G, Oudot-Le Secq M-P, Stam WT, Olsen JL. 2006 A mtDNA-based phylogeny of the brown algal genus *Fucus* (Heterokontophyta; Phaeophyta). *Mol. Phylogenet. Evol.* **39**, 209–222. (doi:10.1016/j.ympev.2006.01.019)
41. Hoarau G, Coyer JA, Veldsink JH, Stam WT, Olsen JL. 2007 Glacial refugia and recolonization patterns in the brown seaweed *Fucus serratus*. *Mol. Ecol.* **16**, 3606–3616. (doi:10.1111/j.1365-294X.2007.03408.x)
42. Serrão EA, Alice LA, Brawley SH. 1999 Evolution of the Fucaeae (Phaeophyceae) inferred from nrDNA-ITS. *J. Phycol.* **35**, 382–394. (doi:10.1046/j.1529-8817.1999.3520382.x)
43. Coyer JA, Hoarau G, Costa JF, Hogerdijk B, Serrão EA, Billard E, Valero M, Pearson G, Olsen JL. 2011 Evolution and diversification within the intertidal brown macroalgae *Fucus spiralis*/*F. vesiculosus* species complex in the North Atlantic. *Mol. Phylogenet. Evol.* **58**, 283–296. (doi:10.1016/j.ympev.2010.11.015)
44. Cánovas F, Mota C, Serrão E, Pearson GA. 2011 Driving south: a multi-gene phylogeny of the brown algal family Fucaeae reveals relationships and recent drivers of a marine radiation. *BMC Evol. Biol.* **11**, 371–376. (doi:10.1186/1471-2148-11-371)
45. Gladenkov AV, Oleinik AE, Marinovich LJ, Barinov KB. 2002 A refined age for the earliest opening of the Bering Strait. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **183**, 321–328. (doi:10.1016/S0031-0182(02)00249-3)
46. Coyer JA, Hoarau G, Stam WT, Olsen JL. 2007 Hybridization and introgression in a mixed population of the intertidal seaweeds *Fucus distichus* and *F. serratus*. *J. Evol. Biol.* **16**, 3606–3616.
47. Coyer JA, Peters AF, Hoarau G, Stam WT, Olsen JL. 2002 Hybridisation of the marine seaweeds, *Fucus serratus* and *F. evanescens* (Heterokontophyta; Phaeophyceae) in a century-old zone of secondary contact. *Proc. R. Soc. Lond. B* **269**, 1829–1834. (doi:10.1098/rspb.2002.2093)
48. Wallace A, Klein AS, Mathieson AC. 2004 Determining the affinities of salt marsh furoids using microsatellite markers: evidence of hybridization and introgression between two species of *Fucus* (Phaeophyta) in a Maine estuary. *J. Phycol.* **40**, 1013–1027. (doi:10.1111/j.1529-8817.2004.04085.x)
49. Billard E, Daguin C, Pearson GA, Serrão E, Engel CR, Valero M. 2005 Genetic isolation between three closely related taxa: *Fucus vesiculosus*, *F. spiralis* and *F. ceranoides*. *J. Phycol.* **41**, 900–905. (doi:10.1111/j.0022-3646.2005.04221.x)
50. Engel CR, Daguin C, Serrão E. 2005 Genetic entities and mating system in hermaphroditic *Fucus spiralis* and its close dioecious relative *F. vesiculosus* (Fucaeae, Phaeophyceae). *Mol. Ecol.* **14**, 2033–2046. (doi:10.1111/j.1365-294X.2005.02558.x)
51. Coyer JA, Hoarau G, Pearson G, Serrão E, Stam WT, Olsen JL. 2006 Convergent adaptation to a marginal habitat by homoploid hybrids and polyploid ecads in the seaweed genus *Fucus*. *Biol. Lett.* **2**, 405–408. (doi:10.1098/rsbl.2006.0489)
52. Mathieson AC, Dawes CJ, Wallace A, Klein AS. 2006 Distribution, morphology, and genetic affinities of dwarf embedded *Fucus* populations from the northwest Atlantic Ocean. *Bot. Mar.* **49**, 283–303. (doi:10.1515/BOT.2006.036)
53. Müller DG, Gassman G. 1984 Sexual reproduction and the role of sperm attractants in monoecious species of the brown algae order Fucales (*Fucus*, *Hesperophycus*, *Pelevita*, and *Pelvetiopsis*). *J. Plant Physiol.* **118**, 401–408. (doi:10.1016/S0176-1617(85)80200-5)
54. Arrontes J. 1993 Nature of the distributional boundary of *Fucus serratus* on the north shore of Spain. *Mar. Ecol. Prog. Ser.* **93**, 183–193. (doi:10.3354/meps093183)
55. Serrão EA, Kautsky L, Lifvergren T, Brawley S. 1997 Gamete dispersal and pre-recruitment mortality in Baltic *Fucus vesiculosus*. *Phycol. Suppl.* **36**, 101–102.
56. Sideman EJ, Mathieson AC. 1983 Ecological and genealogical distinctions of a high intertidal, dwarf form of *Fucus distichus* (L.) Powell. *J. Exp. Mar. Biol. Ecol.* **72**, 171–188. (doi:10.1016/0022-0981(83)90142-9)
57. Sideman EJ, Mathieson AC. 1985 Morphological variation within and between natural populations of non-tide pool *Fucus distichus* (Phaeophyta) in New England. *J. Phycol.* **21**, 250–257. (doi:10.1111/j.0022-3646.1985.00250.x)
58. Kucera H, Saunders GW. 2008 Assigning morphological variants of *Fucus* (Fucales, Phaeophyceae) in Canadian waters to recognized species using DNA barcoding. *Botany* **86**, 1065–1079. (doi:10.1139/B08-056)
59. Coyer JA, Hoarau G, Van Schaik J, Luijckx P, Olsen JL. 2011 Trans-Pacific and trans-Arctic pathways of the intertidal macroalgal *Fucus distichus* L. reveal multiple glacial refugia and colonisations from the North Pacific to the North Atlantic. *J. Biogeogr.* **38**, 756–771. (doi:10.1111/j.1365-2699.2010.02437.x)
60. Coyer JA, Hoarau G, Skage M, Stam WT, Olsen JL. 2006 Origin of *Fucus serratus* (Heterokontophyta; Fucaeae) populations on Iceland and The Faroes: a microsatellite-based assessment. *Eur. J. Phycol.* **41**, 235–246. (doi:10.1080/09670260600652820)
61. Schueller GH, Peters AF. 1994 Arrival of *Fucus evanescens* (Phaeophyceae) in Kiel Bight (Western Baltic). *Bot. Mar.* **37**, 471–477. (doi:10.1515/botm.1994.37.5.471)
62. Wikström SA, von Wachenfeldt T, Kautsky L. 2002 Establishment of the exotic species *Fucus evanescens* C. Ag. (Phaeophyceae) in Øresund, southern Sweden. *Bot. Mar.* **45**, 510–517. (doi:10.1515/BOT.2002.054)
63. Coyer JA, Peters AF, Hoarau G, Stam WT, Olsen JL. 2002 Inheritance patterns of ITS1, chloroplasts, and mitochondria in artificial hybrids of the marine rockweeds, *Fucus serratus* and *F. evanescens* (Heterokontophyta; Fucaeae). *Eur. J. Phycol.* **37**, 173–178. (doi:10.1017/S0967026202003682)
64. Nei M. 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590.
65. Wright S. 1969 *Evolution and the genetics of populations, vol. 2: the theory of gene frequencies*. Chicago, IL: University of Chicago Press.
66. Weir BS, Cockerham CC. 1984 Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370. (doi:10.2307/2408641)
67. Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F. 2001 *GENETIX, logiciel sous Windows pour la génétique des populations*, 4.02 edn. Montpellier, France: Laboratoire Genome et populations.
68. Pritchard JK, Stephens P, Donnelly P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.
69. Nielsen EEG, Bach LA, Kotlicki P. 2006 HYBRIDLAB (version 1.0): a program for generation simulated hybrids from population samples. *Mol. Ecol. Notes* **6**, 971–973. (doi:10.1111/j.1471-8286.2006.01433.x)
70. Lobban CS, Harrison PJ. 1994 *Seaweed ecology and physiology*, p. 366. New York, NY: Cambridge University Press.
71. Sasa MM, Chippindale PT, Johnson NA. 1998 Patterns of postzygotic isolation in frogs. *Evolution* **52**, 1811–1820. (doi:10.2307/2411351)
72. Presgraves DC. 2002 Patterns of postzygotic isolation in Lepidoptera. *Evolution* **56**, 1168–1183. (doi:10.1111/j.0014-3820.2002.tb01430.x)
73. Fredriksen S. 1990 Fenologiske undersøkelser av tangarter fra Sør-Norge og Nord-Norge. *Blyttia* **48**, 3–7.
74. Lessios HA. 2007 Reproductive isolation between species of sea urchins. *Bull. Mar. Sci.* **81**, 191–208.
75. Slaughter C, McCartney MA, Yund PO. 2008 Comparison of gamete compatibility between two blue mussel species in sympatry and in allopatry. *Biol. Bull.* **214**, 57–66. (doi:10.2307/25066660)
76. Palumbi SR. 2009 Speciation and the evolution of gamete recognition genes: pattern and process. *Heredity* **102**, 66–76. (doi:10.1038/hdy.2008.104)
77. Lessios HA. 2011 Speciation genes in free-spawning marine invertebrates. *Integr. Comp. Biol.* **51**, 456–465. (doi:10.1093/icb/icr039)
78. Metz EC, Palumbi SR. 1996 Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. *Mol. Biol. Evol.* **13**, 397–406. (doi:10.1093/oxfordjournals.molbev.a025598)
79. Riginos C, McDonald JH. 2003 Positive selection on an acrosomal sperm protein, M7 lysin, in three species of the mussel genus *Mytilus*. *Mol. Biol. Evol.* **20**, 200–207. (doi:10.1093/molbev/msg021)
80. Zigler KS, Lessios HA. 2003 Evolution of bindin in the pantropical sea urchin *Tripeustes*: comparisons to bindin of other genera. *Mol. Biol. Evol.* **20**, 220–231. (doi:10.1093/molbev/msg020)
81. Metz EC, Gomez-Gutierrez G, Vacquier VD. 1998 Mitochondrial DNA and bindin gene sequence evolution among allopatric species of the sea urchin genus *Arbacia*. *Mol. Biol. Evol.* **15**, 185–195. (doi:10.1093/oxfordjournals.molbev.a025914)
82. Wright PJ, Green JR, Callow JA. 1995 The *Fucus* (Phaeophyceae) sperm receptor for eggs. 1. Development and characteristics of a binding assay. *J. Phycol.* **31**, 584–591. (doi:10.1111/j.1529-8817.1995.tb02554.x)
83. Wright PJ, Callow JA, Green JR. 1995 The *Fucus* (Phaeophyceae) sperm receptor for eggs. 2. Isolation of a binding-protein which partially activates eggs. *J. Phycol.* **31**, 592–600. (doi:10.1111/j.1529-8817.1995.tb02555.x)
84. Callow JA, Stafford CR, Wright PJ, Green JR. 1994 Glycoprotein domains and their role in gamete recognition in *Fucus*. *Biochem. Soc. Symp.* **60**, 35–41.
85. Gavrilov S, Waxman D. 2002 Sympatric speciation by sexual conflict. *Proc. Natl Acad. Sci. USA* **99**, 10 533–10 538. (doi:10.1073/pnas.152011499)
86. Haygood R. 2004 Sexual conflict and protein polymorphism. *Evolution* **58**, 1414–1423. (doi:10.1111/j.0014-3820.2004.tb01723.x)
87. Levitan DR, Stappert AP. 2010 Simultaneous positive and negative frequency-dependent selection on

- sperm bindin, a gamete recognition protein in the sea urchin *Strongylocentrotus purpuratus*. *Evolution* **64**, 785–797. (doi:10.1111/j.1558-5646.2009.00850.x)
88. Brawley SH. 1987 A sodium-dependent, fast block to polyspermy occurs in eggs of furoid algae. *Dev. Biol.* **124**, 390–397. (doi:10.1016/0012-1606(87)90491-X)
 89. Brawley SH. 1991 The fast block against polyspermy in furoid algae is an electrical block. *Dev. Biol.* **144**, 94–106. (doi:10.1016/0012-1606(91)90482-I)
 90. Serrão E, Kautsky L, Brawley S. 1999 Reproductive success in *Fucus vesiculosus* L. in the brackish Baltic Sea correlates with osmotic capabilities of Baltic gametes. *J. Phycol.* **35**, 254–269. (doi:10.1046/j.1529-8817.1999.3520254.x)
 91. Brawley S. 1992 Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. *Mar. Biol.* **113**, 145–157. (doi:10.1007/BF00367648)
 92. Pearson G, Brawley S. 1996 Reproductive ecology of *Fucus distichus* (Phaeophyceae): an intertidal alga with successful external fertilization. *Mar. Ecol. Prog. Ser.* **143**, 211–223. (doi:10.3354/meps143211)
 93. Pereyra RT, Bergström L, Kautsky L, Johannesson K. 2009 Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. *BMC Evol. Biol.* **70**, 1–9.